Small-Diameter Blood Vessels Engineered With Bone Marrow–Derived Cells

Seung-Woo Cho, MS,[†] Sang Hyun Lim, MD,[‡] Il-Kwon Kim, MS,[‡§] Yoo Sun Hong, MD,[‡] Sang-Soo Kim, MS,[*‖] Kyung Jong Yoo, MD,[‡] Hyun-Young Park, MD, PhD,[¶] Yangsoo Jang, MD, PhD,[¶] Byung Chul Chang, MD, PhD,[‡] Cha Yong Choi, PhD,[†‖] Ki-Chul Hwang, PhD,[**] and Byung-Soo Kim, PhD[*]*

Objective: The objective of this study is to investigate if bone marrow–derived cells (BMCs) regenerate vascular tissues and improve patency in tissue-engineered small-diameter (internal diameter = 3 mm) vascular grafts.

Summary Background Data: BMCs have demonstrated the ability to differentiate into endothelial-like cells and vascular smooth muscle–like cells and may offer an alternative cell source for vascular tissue engineering. Thus, we tissue-engineered small-diameter vascular grafts with BMCs and decellularized arteries.

Methods: Canine BMCs were differentiated in vitro into smooth muscle α-actin/smooth muscle myosin heavy-chain-positive cells and von Willebrand factor/CD31-positive cells and seeded onto decellularized canine carotid arteries (internal diameter = 3 mm). The seeded grafts were implanted in cell donor dogs. The vascular-tissue regeneration and graft patency were investigated with immunohistochemistry and angiography, respectively.

Results: The vascular grafts seeded with BMCs remained patent for up to 8 weeks in the canine carotid artery interposition model, whereas nonseeded grafts occluded within 2 weeks. Within 8 weeks after implantation, the vascular grafts showed regeneration of the 3 elements of artery (endothelium, media, and adventitia). BMCs labeled with a fluorescent dye prior to implantation were detected in the retrieved vascular grafts, indicating that the BMCs participated in the vascular tissue regeneration.

Conclusions: Here we show that BMCs have the potential to regenerate vascular tissues and improve patency in tissue-engineered small-diameter vascular grafts. This is the first report of a small-diameter neovessel engineered with BMCs as a cell source.

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Therapies for coronary artery disease and peripheral vascular disease often require replacement of the diseased vessels with vascular grafts. Autologous arteries or veins are the best substitutes for small-diameter (internal diameter [ID] < 6 mm) vessels.[1] However, many patients do not have vessels suitable for grafting due to preexisting vascular diseases or vessel use in previous procedures. Therefore, there have been attempts to develop a small-diameter vascular graft made of synthetic or natural polymers. The synthetic polymeric materials include polyethylene terephthalate and expanded polytetrafluoroethylene (ePTFE).[2] Although these polymeric vascular grafts have been successfully employed to replace blood vessels above 6 mm in ID, these polymeric grafts cannot be used for treatment of small-diameter vascular diseases due to thrombus formation.[3,4] Coating of the intimal side with antithrombogenic materials, such as heparin,[5] polyethylene oxide,[6] or endothelial cells (ECs),[7] has been applied to solve this problem, but these approaches were unsuccessful. A decellularized tissue matrix has been shown to be successfully remodeled into cellularized vessels after implantation in rabbits.[8] However, this result may not be relevant to humans, because endothelial-cell development on vascular grafts in humans is much slower than in rabbits.[9]

Recently, tissue-engineering techniques have been applied to develop small-diameter vascular grafts. Several studies have fabricated small-diameter vascular grafts by culturing smooth muscle cells (SMCs) and ECs,[10–12] which were

From the *Department of Chemical Engineering, Hanyang University, Seoul 133-791, Korea; †School of Chemical Engineering, Seoul National University, Seoul 151-742, Korea; ‡Division of Cardiovascular Surgery, Cardiovascular Center, Yonsei University College of Medicine, Seoul 120-752, Korea; §Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, Korea; ‖Interdisciplinary Program for Biochemical Engineering and Biotechnology, Seoul National University, Seoul 151-742, Korea; ¶Division of Cardiology, Cardiovascular Center, Yonsei University College of Medicine, Seoul 120-752, Korea; and the **Cardiovascular Research Institute, Yonsei University College of Medicine, Seoul 120-752, Korea.

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Seung-Woo Cho and Sang Hyun Lim contributed equally to this work.

Reprints: Byung-Soo Kim, PhD, Department of Chemical Engineering, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 133-791, Korea. E-mail: bskim@hanyang.ac.kr.

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isolated from autologous blood vessels, either on biodegradable polymer scaffolds or without exogenous materials, but these grafts developed thrombosis shortly after implantation.[11,12] Another drawback of this approach is that the graft fabrication procedures involve a blood vessel biopsy to obtain vascular cell sources, which may result in potential morbidity at the donor sites and could be limited by a healthy autologous vessel shortage. Another cell source for tissue engineering of vascular grafts is multipotent stem cell. Recently, vascular grafts fabricated by seeding endothelial progenitor cells (EPCs) derived from peripheral blood onto decellularized vessels were shown to maintain patency for 130 days in the ovine model.[13] However, seeding only with EPCs would not induce complete smooth muscle (SM) regeneration in the arterial grafts.

Bone marrow–derived cells (BMCs) could be an alternative stem cell source for vascular tissue engineering. BMCs have demonstrated the ability to differentiate into multiple mesenchymal cell lineages.[14,15] Recently, BMCs have been shown to differentiate into endothelial-like cells[16–19] and vascular SM-like cells.[20–23] Furthermore, several studies have demonstrated that bone marrow mononuclear cells (BMMNCs) contribute to histogenesis of tissue-engineered large-diameter vascular grafts[24] and that bone marrow CD34-positive cells enhance the graft endothelialization in vivo.[25]

In this study, we attempted to develop small-diameter (ID = 3 mm) vascular grafts using BMCs and showed that BMC seeding significantly improved the patency of tissue-engineered small-diameter vascular grafts. Small-diameter blood vessels were fabricated by seeding 2 types of vascular cells differentiated from autologous BMMNCs onto decellularized carotid arteries and implanted in canine models. Prior to cell seeding, BMCs were induced to differentiate in vitro into SM α-actin/SM myosin heavy-chain (SMMHC)–positive cells and von Willebrand factor (vWF)/CD31-positive cells by culturing BMCs in different types of media. Decellularized arteries were used as scaffolds for vascular tissue reconstruction because these scaffolds preserved biochemical components and structures of native extracellular matrices (ECMs) of blood vessels. The vascular tissue regeneration and graft patency were examined.

MATERIALS AND METHODS

Preparation of Decellularized Vascular Matrix

Decellularized vascular matrices (3 mm in ID, 40 mm in length) were prepared as previously described.[13] In brief, freshly harvested canine carotid arteries were washed with distilled water for 1 hour to remove blood elements. The vessels were then immersed in a decellularization solution (0.5% (vol/vol) Triton X-100 (Sigma, St. Louis, MO) and 0.05% (vol/vol) ammonium hydroxide (Sigma) in distilled water) with shaking at 4°C for 3 days. To remove the residual detergent, the decellularized vessels were washed in distilled water with shaking at 4°C for 3 days. The resultant matrices were lyophilized for 1 day and sterilized with ethylene oxide gas at room temperature.

BMC Isolation and Culture

Mongrel dogs (20–25 kg) were anesthetized, and bone marrow (30 mL for each dog) was aspirated from the humeri of the dogs and immediately mixed with heparin (100 unit heparin/mL bone marrow). The mixture was centrifuged on a Ficoll-Paque density gradient (Amersham Bioscience, Arlington Heights, IL) for 20 minutes at 1500 rpm. BMMNCs were isolated from the buffy coat layer between the Ficoll-Paque reagent and blood plasma component and washed 3 times in phosphate-buffered saline (PBS; Sigma) solution. BMMNC fraction for SM α-actin/SMMHC-positive cells was cultured in Medium 199 (Gibco BRL, Gaithersburg, MD) containing 10% (vol/vol) fetal bovine serum (FBS), 1% (wt/vol) penicillin and streptomycin (Gibco BRL). BMMNC fraction for vWF/CD31-positive cells was cultured in EBM-2 (Clonetics, San Diego, CA) supplemented with human vascular endothelial growth factor (VEGF; 10 ng/mL; PeproTech, Rocky Hill, NJ), human basic fibroblast growth factor (bFGF; 2 ng/mL; PeproTech), human epidermal growth factor (10 ng/mL; PeproTech), human insulin-like growth factor-1 (5 ng/mL; PeproTech), and ascorbic acid on culture dishes coated with 1 μg/cm^2 human fibronectin (Sigma). The culture medium was changed every 2 days.

BMC Characterization

Cultured BMCs were stained immunohistochemically using antibodies against SM α-actin (DAKO, Carpenteria, CA), SMMHC (DAKO), vWF (DAKO), and CD31 (DAKO). The staining signal was developed with avidin-peroxidase system (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, CA).

BMC Labeling

Cultured BMCs were labeled with 1 μg/mL of Cell Tracker chloromethyl-1,1′-dioactadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (CM-DiI; Molecular Probes, Eugene, OR), a fluorescent carbocyanine dye, at 37°C for 5 minutes and then at 4°C for 15 minutes. The labeled cells were washed 3 times with PBS and seeded onto decellularized vascular matrices.

Cell Seeding Onto Vascular Matrix and in Vitro Maintenance

SM α-actin/SMMHC-positive cells and vWF/CD31-positive cells differentiated from BMCs were cultured for 3 weeks to obtain enough cells for seeding onto the vascular grafts. The cultured BMCs were passaged twice for 3-week culture. SM α-actin/SMMHC-positive cells were uniformly seeded onto small-diameter decellularized matrices at a cell

density of 3×10^7 cells/mL. Two hours later, vWF/CD31-positive cells were then uniformly seeded onto the luminal sides of the matrices at a cell density of 1×10^7 cells/mL. The seeded grafts were maintained in vitro in Medium 199 supplemented with 20% (vol/vol) FBS, VEGF (10 ng/mL), and bFGF (2 ng/mL) for 1 week prior to implantation. The medium was changed every day. Intraluminal mechanical supports for prevention of the graft lumen collapse were not used.

Scanning Electron Microscopy

The explanted grafts were fixed in 1% (vol/vol) buffered glutaraldehyde and 0.1% (vol/vol) buffered formaldehyde for 30 minutes and 24 hours, respectively, dehydrated with a graded ethanol series, and dried. The dried samples were mounted on aluminum stub and sputter-coated with gold. Scanning electron microscope (JSM-6330F; JEOL, Tokyo, Japan) was used to image the samples.

Suture Retention Strength Measurement

The suture retention strength of the vascular grafts was measured using an Instron mechanical tester (Instron 4465; Instron, Canton, MA) as previously described.[26] One end of the specimen was fixed by stage clamp of Instron tester, and the other end was connected to another clamp by a suture material (4–0 prolene; Ethicon, Somerville, NJ). The measurement was performed using a 100-Newton maximum load cell, and the cross-head speed was 10 mm/min. A tensile force was applied until the grafts were completely torn off and the graft rupture stress was recorded.

DNA Quantification

To determine whether the cellular components were completely removed from the canine carotid arteries through the decellularization process, the total DNA in the decellularized matrices was quantified. DNA from tissues was isolated using a Wizard Genomic DNA Purification kit (Promega, Madison, WI). The DNA content was determined with ultraviolet absorbance spectrophotometry at 260 nm and compared with the DNA content in the native canine carotid arteries.

Surgical Implantation of Vascular Grafts

Bone marrow donor mongrel dogs (20–25 kg) were anesthetized with injection of intramuscular ketamine (30 mg/kg) and intravenous pentobarbital (30 mg/kg) and ventilated with a mixture of O_2, N_2, and isoflurane during the operation. Through a longitudinal midneck incision, common carotid arteries were exposed. Prior to arterial clamping, heparin (100 unit/kg; Choongwae Pharma Co, Seoul, Korea) was administered intravenously. The grafts were placed as an end-to-end anastomosis to the common carotid arteries using a 6–0 Prolene suture (Ethicon). Decellularized grafts without cell seeding served as controls. Prior to implantation, the control grafts were rehydrated in PBS solution. The arterial flow was reestablished and the closure was sutured by layers. No anticoagulants or antiplatelets were administered postoperatively. The implanted graft patency was monitored by the arterial digital subtraction angiography every week. All care and handling of the animals were provided according to the Guide for the Care and Use of Laboratory Animals of Yonsei University.

Explant Characterization

Midportion segments of the grafts were fixed with 10% (vol/vol) buffered formaldehyde solution and dehydrated with a series of ethanol. The samples were embedded in paraffin, sectioned 4 μm in thickness, and stained with hematoxylin and eosin (H&E). Elastin and collagen in the explant tissue sections were stained with van Gieson method and Masson trichrome method, respectively. The tissue sections were also stained immunohistochemically for vWF, SM α-actin, and SMMHC using avidin-peroxidase system (Vectastain Elite ABC kit). CM-DiI-labeled cells in the tissue sections were analyzed using a fluorescence microscope (Eclipse E800; Nikon, Tokyo, Japan) before and after implantation.

Statistical Analysis

Result data were expressed as mean ± SD. Statistical analysis was performed by unpaired Student t test using InStat software (InStat 3.0; GraphPad Software Inc, San Diego, CA). A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Decellularized Vascular Matrices

Decellularized canine carotid arteries 3 mm in ID and 40 mm in length were used as the vascular matrices (Fig. 1A). Decellularized matrices were prepared by removing cellular components from the canine carotid arteries and leaving the native ECMs of the arteries. H&E staining revealed that the vascular matrices processed with the decellularization technique were acellular (Fig. 1B). Histologic analysis (van Gieson staining) confirmed that ECMs, such as internal elastic lamina and external elastic lamellae, were well preserved in the decellularized vascular matrices (Fig. 1C). The matrices exhibited porous structures (Fig. 1D), which would be appropriate for cell seeding and adhesion. Endothelium was not observed in the scanning electron micrographs (Fig. 1E). Mercury porosimetric analysis revealed that the porosity of the decellularized matrices was 66.8%. The pore size was mainly below 20 μm in diameter (Fig. 1F). The microporous structures of the matrices would be appropriate for migration and ingrowth of seeded cells. Quantification of DNA content in the decellularized arteries showed that almost all the cellular components in the matrices were removed by the

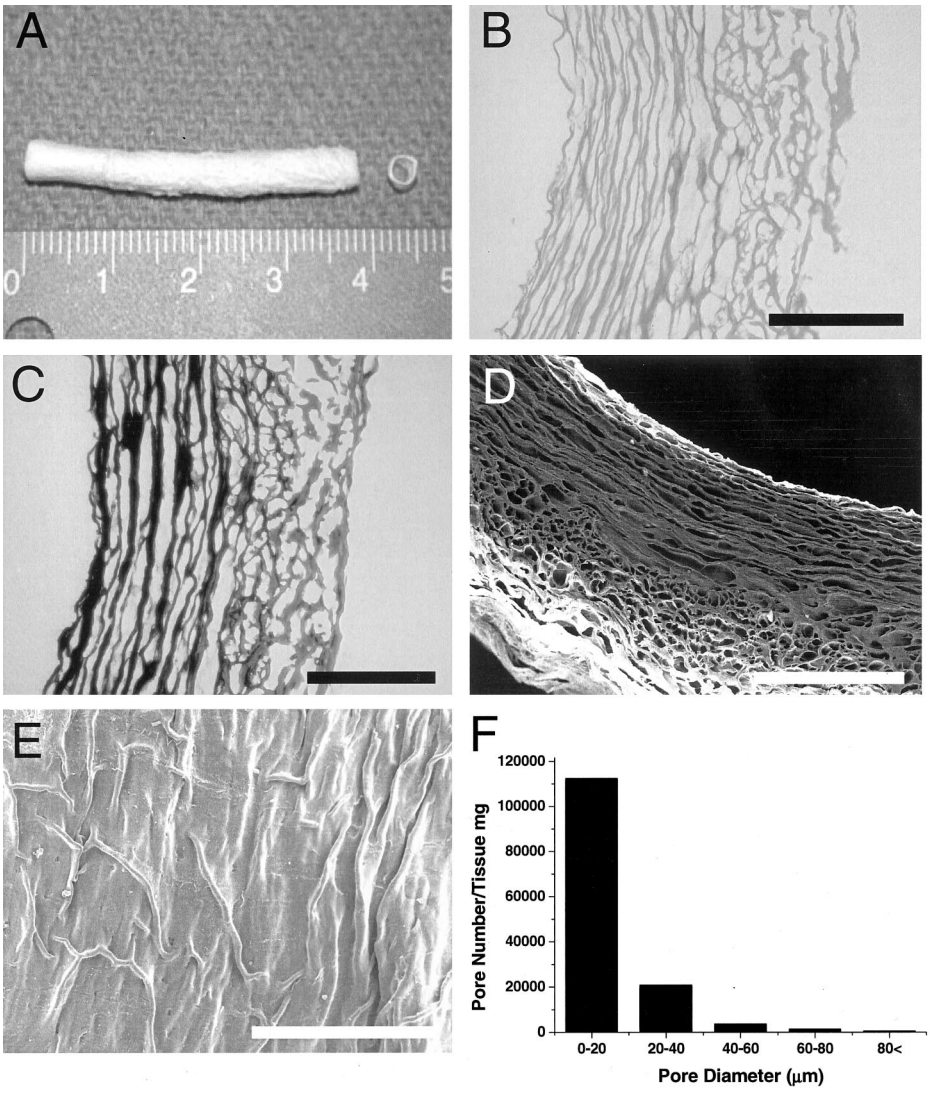

FIGURE 1. Characterization of a decellularized vascular matrix. A, A gross view of decellularized canine carotid artery (length = 40 mm, ID = 3 mm). The scale is in centimeters. B, H&E staining showed complete removal of cellular components from canine carotid artery (× 100). C, van Gieson elastin staining showed well-preserved elastin layers in the decellularized matrix (× 100). D, Scanning electron micrograph of the cross-section of the matrix (× 200). E, Scanning electron micrograph of the luminal surface of the matrix (× 250). The scale bars indicate 200 μm. F, Pore size distribution of the decellularized matrix as determined by mercury porosimetry. The average pore diameter of the matrix was approximately 17.3 μm.

nonionic detergent treatment, as the DNA content in the decellularized matrices was only 1.7% of that in the native carotid arteries.

Vascular Cells from Bone Marrow

To differentiate BMCs into vWF/CD31-positive cells and SM α-actin/SMMHC-positive cells, BMCs were cultured in each medium appropriate for either endothelial-like cell or vascular SM-like cell differentiation. After 3 weeks in culture, the BMCs cultured in the medium for endothelial-like cells showed cobblestone morphology, a characteristic of ECs (Fig. 2A). In addition, these cells expressed vWF (Fig. 2B) and CD31 (Fig. 2C). The BMCs cultured in the medium for SM-like cells showed SMC-like morphology (Fig. 2D) and stained positively for SM α-actin (Fig. 2E) and SMMHC (Fig. 2F). These results demonstrate that endothelial-like and SM-like cells for vascular tissue engineering can be obtained from bone marrow.

Cell Seeding Onto Decellularized Vascular Matrices and in Vitro Maintenance

Vascular cells derived from bone marrow were seeded onto decellularized vascular matrices. BMCs cultured in the medium for endothelial-like cells and medium for SM-like cells were seeded onto intimal sides and outsides of the decellularized vascular matrices, respectively. After seeding, the seeded vascular grafts (Fig. 3A) were maintained in vitro for 1 week to provide the cell adhesion to the grafts. Scanning electron micrographic examination of the luminal surfaces of the grafts revealed a confluent layer of endothelial-like cells (Fig. 3B). Histologic cross-sections of the grafts confirmed

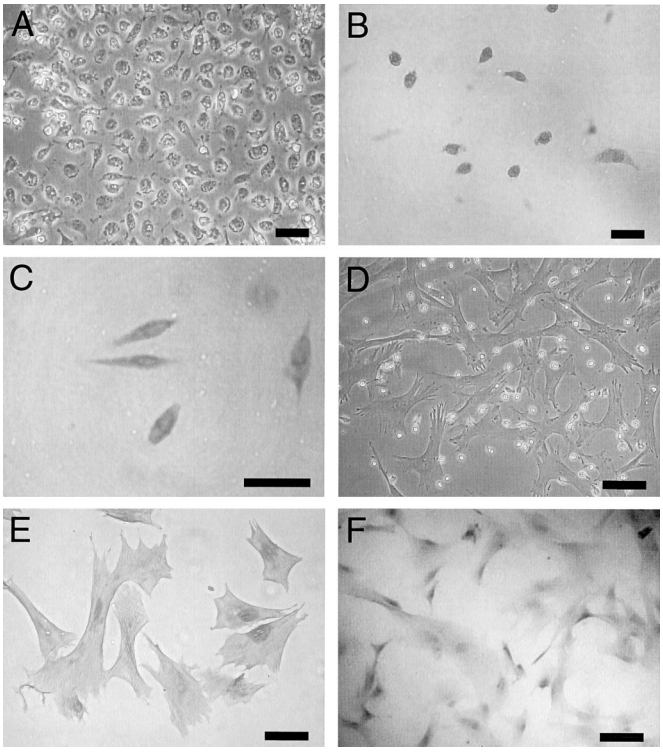

FIGURE 2. Characterization of cultured bone marrow–derived cells (BMCs). A, BMCs expanded in endothelial-like cell culture condition showed cobblestone morphology of typical ECs (× 100). Cultured endothelial-like cells stained positively for (B) vWF (× 100) and (C) CD31 (× 400), which are EC markers. D, BMCs expanded in SM-like cell culture condition had morphology similar to that of mature SMCs (× 100). Cultured SM-like cells expressed (E) SM α-actin (× 100) and (F) SMMHC (× 100), which are SMC markers. The scale bars indicate 10 μm.

that most of the seeded cells were distributed near the intima and adventitia of the vascular grafts (Fig. 3C).

Suture Retention Strength

To evaluate in vivo effectiveness of the vascular grafts, implantation studies were performed in canine models. Prior to implantation, the suture retention strength of the vascular grafts was measured to determine whether the grafts could withstand forces exerted in anastomosis. The average suture retention strength of the vascular grafts was 606 ± 98 g, and it was not statistically different from that of the native canine carotid arteries (753 ± 112 g) ($P > 0.05$) (Fig. 4), suggesting that the vascular graft has sufficient suture retention strength to withstand anastomosis forces. Practically, the vascular grafts were anastomosed to carotid arteries in canine models, without graft rupture (Fig. 5).

Graft Patency

Segments of the carotid arteries in bone marrow donor dogs were replaced by seeded (n = 6, autologous) and unseeded (n = 6, controls) vascular grafts (40 mm in length and 3 mm in ID) using the end-to-end anastomosis. To determine the graft patency, the animals were periodically investigated by arterial digital subtraction angiography after implantation (Fig. 6A, B). Patency of the vascular grafts was significantly improved by BMC seeding. Out of the 6 unseeded control grafts, 4 and 2 grafts occluded by 1 and 2 weeks, respectively, with thrombus formation (Fig. 6C). In contrast, the vascular graft seeded with autologous BMCs maintained patency for up to 8 weeks (Fig. 6C).

Vascular Tissue Regeneration in vivo

Histologic analyses of the seeded vascular grafts at 8 weeks revealed regeneration of the 3 elements of artery (endothelium, media, and adventitia). The H&E-stained sections showed reconstruction of the artery, with structures very similar to those of the native artery (Fig. 7A). Two major ECMs in the artery were present in the seeded vascular grafts at 8 weeks. Elastin staining by van Gieson method displayed internal elastic lamina and external elastic lamellae layers in the vascular grafts (Fig. 7B). A significant amount of collagen was present in the grafts, as evaluated by Masson trichrome staining (Fig. 7C). The cells in the media stained positively for SM α-actin and SMMHC (Fig. 7D, E), both of which are characteristics of SMCs, indicating regeneration of SM in the media. The cells lining the lumen stained positively for vWF, which is a characteristic of ECs (Fig. 7F). Scanning electron microscopic examination also confirmed endothelium regeneration in the grafts (Fig. 7G).

Identification of Implanted BMCs

Next, we investigated if the seeded BMCs were still present on the grafts at 8 weeks. Prior to seeding onto vascular grafts, BMCs were labeled with a fluorescent lipophilic carbocyanine tracer (CM-DiI). Before implantation, the vascular graft sections showed the presence of the labeled BMCs (Fig. 8A). Eight weeks after implantation, the labeled BMCs were found in the intima and media of the vascular grafts (Fig. 8B, C), suggesting that implanted cells survived and participated in the vascular tissue regeneration. Fluorescence intensity diminished probably due to migration of the seeded cells to media and dilution of the cell membrane incorporated dye with cell division.

DISCUSSION

The present study reports the first attempt to develop small-diameter vascular grafts using tissue-engineering technique and bone marrow–derived mesenchymal stem cells. BMCs were induced to differentiate in vitro into SM α-actin/SMMHC-positive cells (SM-like cells) and vWF/CD31-positive cells (endothelial-like cells), and seeded onto decellularized canine carotid arteries. Upon implantation, the vascular grafts showed regeneration of the 3 elements of the

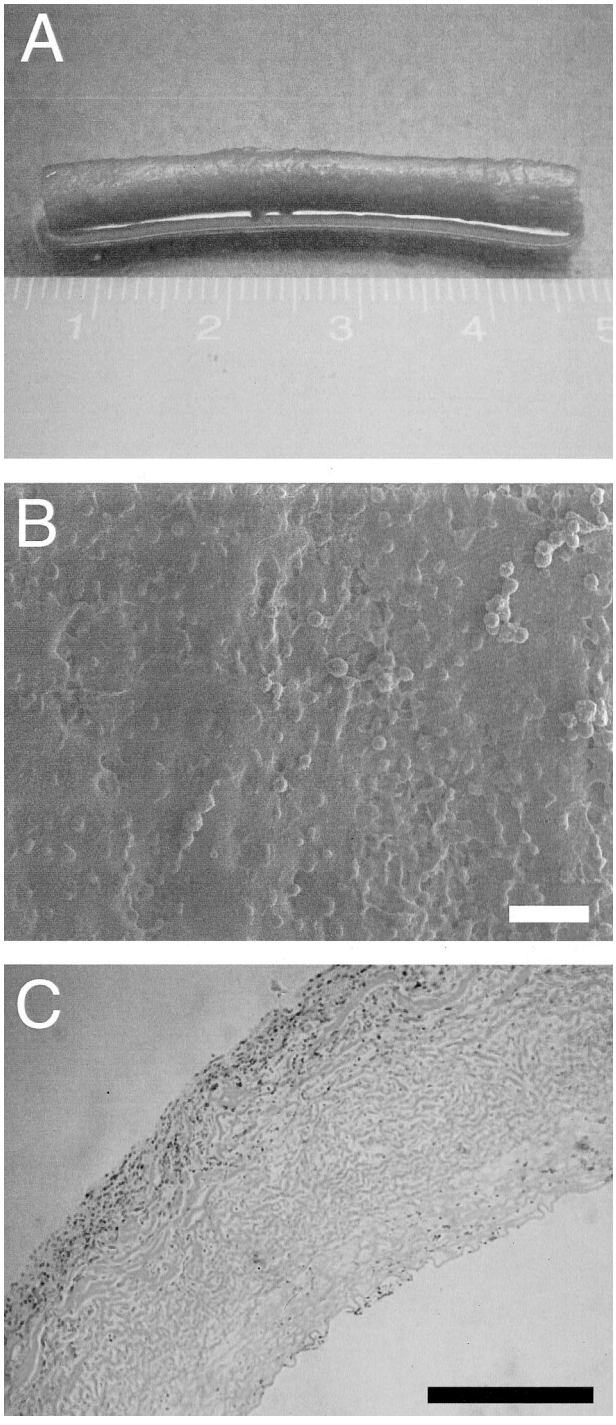

FIGURE 3. Examination of cell-seeded vascular grafts prior to implantation. A, A gross view of a tissue-engineered vascular graft 1 week after BMC seeding. The scale is in centimeters. B, Scanning electron micrograph of the luminal surface of the vascular graft 1 week after cell seeding (× 500). C, H&E staining of the vascular graft 1 week after cell seeding indicated that most of the seeded cells were distributed near the intima and adventitia of the scaffold (× 100). The scale bars in (B) and (C) indicate 30 μm and 200 μm, respectively.

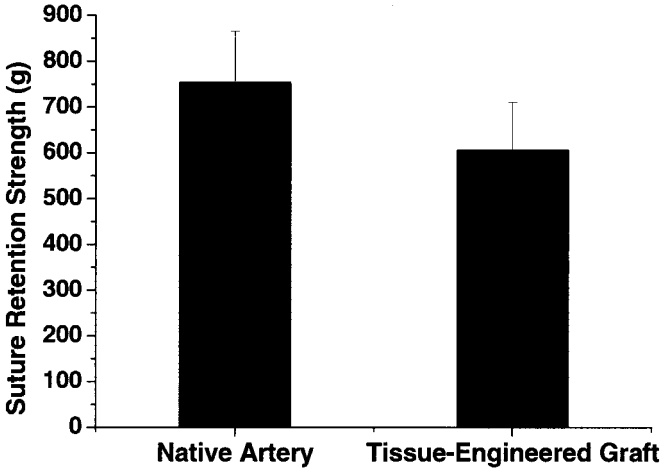

FIGURE 4. Suture retention strength measurement of the tissue-engineered vascular grafts. The average value of the tissue-engineered vascular grafts (n = 4) is slightly lower than that of the native canine carotid arteries (n = 4), but the difference is not statistically significant ($P > 0.05$).

artery (endothelium, media, and adventitia). Importantly, BMC seeding has been found to significantly improve the patency of the tissue-engineered small-diameter (ID = 3 mm) vascular grafts.

Decellularized matrices would be ideal scaffolds for vascular tissue reconstruction. First, decellularized matrices retain native ECMs of blood vessels. Histologic analyses, such as H&E and elastin staining, showed that the structural integrity of the native ECM architecture was well preserved in the matrices (Fig. 1B, C). Decellularization process using

FIGURE 5. Surgical implantation of the tissue-engineered vascular grafts. The grafts were interposed to common carotid arteries by the end-to-end anastomosis in canine models. The arrowheads indicate the anastomotic sites.

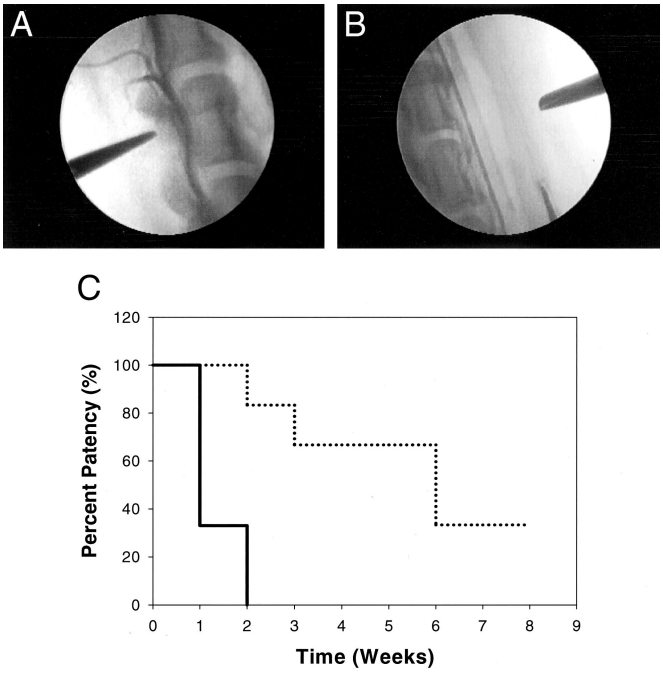

FIGURE 6. Angiogram and patency of the implanted tissue-engineered vascular grafts and control grafts. A, Angiogram of the BMC-seeded vascular grafts 8 weeks after implantation: the grafts maintain patency without occlusion. B, Angiogram of the control grafts (decellularized matrices with no cell seeding) 2 weeks after implantation: the grafts completely occluded within 2 weeks after implantation and showed no blood flow. C, Patency of the implanted tissue-engineered vascular grafts and control grafts. The vascular grafts engineered with BMCs (n = 6, dotted line) maintained patency for up to 8 weeks, whereas all control groups (n = 6, solid line) occluded within 2 weeks.

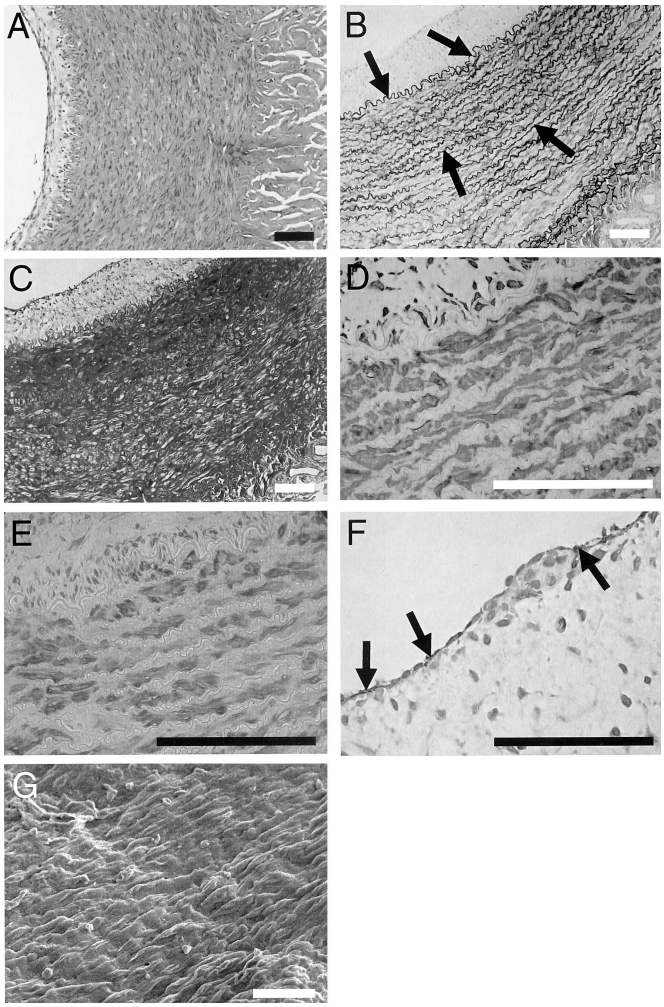

FIGURE 7. Histologic, immunohistochemical, and scanning electron microscopic evaluations of the BMC-seeded vascular grafts retrieved 8 weeks after implantation. A, H&E staining of the retrieved grafts demonstrated regeneration of the 3 elements of artery (endothelium, media, and adventitia) (× 100). B, van Gieson elastin staining showed well-preserved elastin layers. Black wiggly lines are elastin layers (arrows, × 100). C, Masson trichrome staining indicated collagen (blue) between elastin layers (× 100). Immunostaining for (D) SM α-actin (× 400) and (E) SMMHC (× 400) showed the presence of SMCs (brown) in medial layers. F, Cells lining the lumen of the retrieved grafts stained positively for vWF (arrows, brown line, × 400). Scale bars in (A) through (F) indicate 100 μm. G, Scanning electron micrograph of the lumen of the retrieved grafts showed endothelium formation (× 800). The scale bar in (G) indicates 30 μm.

nonionic detergent (Triton X-100), which was used in this study, produces a matrix primarily composed of elastin and insoluble collagen.[27] These structural proteins of decellularized matrix could provide physiologically proper microenvironment for vascular cell repopulation.[28] Through scanning electron microscopic and histologic analyses of the cell-seeded grafts performed prior to implantation, the BMCs were distributed throughout the matrices, and decellularized matrices were confirmed to efficiently work as scaffolds for cell adherence. Second, decellularized matrices may not show immune rejection in vivo. Because cellular components are removed from tissues, the resultant decellularized matrices may not possess antigenic determinants which elicit immune response.[29] Several studies have reported that allogenic decellularized matrices showed low immunogenicity in vivo.[29,30] Third, decellularized matrices have good mechanical properties and handling characteristics. Mechanical properties of artificial vascular grafts are critical for the long-term patency of the grafts.[31] In particular, mismatching of the elasticity between the grafts and the native blood vessels may cause intimal hyperplasia and lead to graft occlusion.[32] A previous study has shown that decellularized porcine carotid

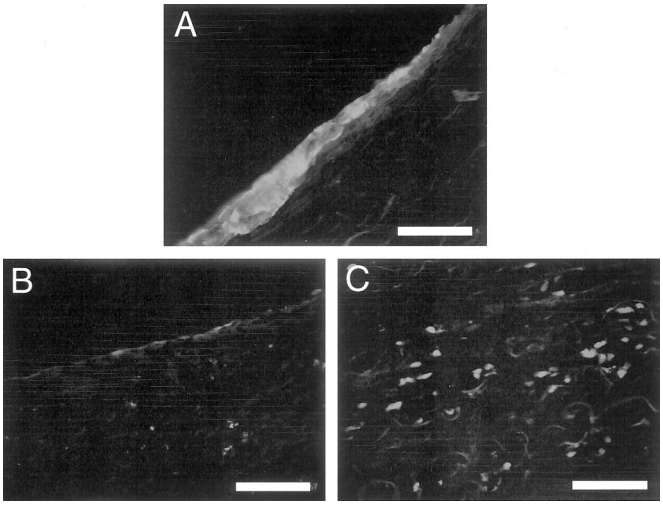

FIGURE 8. Identification of implanted BMCs in the tissue-engineered vascular grafts retrieved 8 weeks after implantation. A, Before implantation, the BMCs labeled with fluorescent cell tracer (CM-DiI) were layered mainly on the luminal sides of the vascular grafts (× 400). The CM-DiI-labeled BMCs were detected on (B) the intimal parts (× 400) and (C) the medial parts (× 400) of the grafts retrieved 8 weeks after implantation. The scale bars indicate 50 μm.

arteries are similar to native arteries in compliance and burst strength.[33] In the present study, the suture retention strength tests have verified that the vascular grafts constructed from decellularized matrices have an appropriate mechanical strength that enables them to endure forces exerted by sutures during surgery, suggesting that they can be anastomosed without graft rupture. The average suture retention strength of the vascular grafts was approximately 600 g, which is considered to be a value sufficient for clinical use. This value is much higher than that of the vascular grafts tissue-engineered from biodegradable synthetic polymers.[12,26] Therefore, decellularized matrices may be more appropriate scaffold for vascular graft than synthetic polymeric matrices. Since the mechanical properties of cell-seeded grafts would be contributed by the ECMs rather than the seeded cells, the mechanical properties of the decellularized matrices would be similar to those of the cell-seeded grafts.

This study showed that the vascular cells exhibiting EC and SMC phenotypes could be induced from BMCs. These results are consistent with those of previous studies demonstrating that endothelial-like and SM-like cells could be derived from BMCs in vitro.[16,17,20,21] There have been evidences from animal studies that BMCs contain endothelial and SM progenitor cells. EPCs mobilized from bone marrow by cytokine induction have been reported to enhance angiogenesis and neovascularization in ischemic tissues.[18,19] In addition, several studies have shown that intimal SMCs contributing to vascular diseases may be derived from BMCs.[22,23] These results suggest that BMCs could be a cell source for EC and SMC.

BMCs would be an ideal cell source for tissue engineering of autologous vascular grafts. Bone marrow aspiration would be less invasive and associated with much lower morbidity at the donor sites than blood vessel biopsy for cell isolation. In addition, BMCs could be used as a cell source when patients do not have blood vessels suitable for biopsy due to preexisting vascular diseases or blood vessel use in previous procedures. Using patients' own BMCs, autologous vascular grafts could be constructed. A clinical case report demonstrated the feasibility of tissue engineering of large-diameter vascular grafts with autologous BMMNCs in humans.[34] In addition, cell therapy using autologous BMMNCs or bone marrow AC133-positive cells has been clinically tested in humans for neovascularization in limb ischemic tissues[35] or cardiac regeneration in infarcted myocardium.[36] These results demonstrate the applicability of BMCs as an autologous cell source in clinical therapy in cardiovascular diseases.

This is the first report of using BMCs for tissue engineering of autologous small-diameter vascular graft. In this study, BMCs implantation onto decellularized vascular matrices (ID = 3 mm) has improved the graft patency significantly and regenerated vascular tissues in vivo. Recent tissue-engineering approaches have also demonstrated the feasibility of using BMCs to construct autologous vascular grafts. Autologous canine BMMNCs seeded onto large-diameter (ID = 8 mm) biodegradable polymer matrices formed vascular tissues in vivo.[24] Nondegradable polymer vascular grafts (ID = 8 mm) seeded with bone marrow CD34-positive cells showed higher endothelialization than nonseeded grafts in a canine model.[25] However, these previous studies have dealt with tissue engineering of large-diameter vascular grafts (ID > 6 mm), and no study has yet reported on engineering of small-diameter vascular grafts with BMCs in vivo.

The patency of the vascular grafts developed in this study needs to be further improved. Although the grafts engineered with BMCs have shown an improved patency compared with nonseeded grafts, occlusion due to thrombus formation has been noted even in grafts engineered with BMCs. The graft occlusion might be due to detachment of endothelial-like cells from the lumen sides of the grafts after implantation in vivo or incomplete endothelialization. It was reported that up to 80% of the seeded ECs were detached from the ePTFE vascular grafts when ECs on the grafts were exposed to blood flow without preconditioning.[37] Cell seeding and in vitro graft maintenance in a static culture condition may be inefficient methods for formation of functional endothelium that can endure in vivo arterial conditions with high shear stress. Dynamic cell seeding on scaffolds[38] and preconditioning of the seeded scaffolds[13,39] in pulsatile bioreactor

may improve functional endothelium formation in vitro and the graft patency in vivo. In addition, several studies have reported that systemic administration of granulocyte colony-stimulating factor (G-CSF) enhanced vascular graft endothelialization in vivo.[40,41] G-CSF has been known to mobilize EPCs in bone marrow and increase EPC numbers in the circulation.[42] Therefore, G-CSF treatment could be considered as a complement to the improvement of the tissue-engineered vascular graft patency.

In summary, this study showed tissue engineering of small-diameter (ID = 3 mm) vascular grafts using BMCs. The vascular grafts engineered with vascular cells (endothelial-like cells and SM-like cells) induced from autologous BMCs showed vascular tissue regeneration and significant patency improvement. Additional studies will be necessary to determine whether the grafts show endothelium functionality, vasoreactivity, and aneurysmal dilation in long-term implantation. Followed by improvement in endothelialization methods for the improved graft patency, this approach may suggest the possibility of constructing functional small-diameter vascular grafts.

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